

Application Serial No. 09/998,333
Amendment dated November 11, 2004
Office Action mailed August 11, 2004

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (currently amended): A method for determining a chemotherapeutic regimen for treating a metastatic tumor in an ~~for an~~ individual having a primary and metastatic tumor, comprising
 - a) obtaining a primary tumor specimen;
 - b) determining expression levels of the EGFR tumor gene determinant in a primary tumor comprising
 - 1) determining mRNA levels of the EGFR tumor gene determinant in the primary tumor sample; and
 - 2) comparing the amount of ~~tumor gene determinant~~ EGFR mRNA levels in the primary tumor sample from step 1) to an amount of mRNA of an internal control gene; and
 - c) determining a chemotherapeutic regimen for treating the metastatic tumor in ~~for the~~ individual based on the amount of ~~tumor gene determinant~~ EGFR mRNA in the primary tumor sample and a predetermined threshold level for the EGFR ~~tumor gene determinant~~.
- 2- 4. (canceled)
5. (currently amended) The method of claim 1 wherein determining expression levels of a EGFR tumor gene determinant comprises a fluorescence based real-time detection method.
- 6-16. (Canceled)
17. (currently amended): A method for determining a chemotherapeutic regimen for treating a metastatic tumor in an individual having a primary and metastatic tumor, comprising
 - a) obtaining a primary tumor specimen and fixing the specimen to obtain a fixed tumor specimen;

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- b) determining a gene expression level for a the EGFR tumor gene determinant in the fixed primary tumor specimen, comprising
 - 1) isolating mRNA for a EGFR ~~tumor gene determinant~~ from the fixed tumor specimen, wherein the fixed tumor sample is heated in the presence of an effective amount of a chaotrophic agent and wherein the heating occurs at a temperature from about 50°C to about 100°C;
 - 2) subjecting the mRNA to amplification using a pair of oligonucleotide primers capable of amplifying a region of the EGFR ~~tumor gene determinant~~ to obtain an amplified sample; and
 - 3) determining the amount of EGFR ~~tumor gene determinant~~ mRNA in the amplified sample;
- c) comparing the amount of EGFR ~~tumor gene determinant~~ mRNA from step 3) to an amount of mRNA of an internal control gene; and
- d) determining a chemotherapeutic regimen for treating the metastatic tumor in the individual based on the amount of ~~tumor gene determinant~~ EGFR mRNA in the primary tumor sample and a predetermined threshold level for the EGFR ~~tumor gene determinant~~.

18-19. (canceled)

20. (currently amended): The method of claim ~~19~~ 17 wherein determining expression levels of a EGFR ~~tumor gene determinant~~ comprises a fluorescence based real-time detection method.

21-23. (canceled)

24. (currently amended): A method for determining a chemotherapeutic regimen for treating a metastatic tumor in an individual having a primary and metastatic tumor, comprising
- a) obtaining a primary tumor specimen;
 - b) fixing and paraffin embedding (FPE) the primary tumor specimen;
 - c) deparaffinizing the tumor specimen to obtain a deparaffinized sample;

- d) determining gene expression levels for ~~a tumor gene determinant~~ EGFR in the deparaffinized sample of the primary tumor specimen, comprising
 - 1) isolating ~~tumor gene determinant~~ EGFR mRNA from the deparaffinized sample, wherein said sample is heated to a temperature in the range of about 50°C to about 100°C; and
 - 2) determining the amount of ~~tumor gene determinant~~ EGFR mRNA by amplifying the mRNA using a pair of oligonucleotide primers capable of ~~capable of~~ amplifying a region of ~~the~~ EGFR ~~tumor gene determinant~~ to obtain an amplified sample;
 - e) comparing the amount of ~~tumor gene determinant~~ EGFR mRNA from step d) to an amount of mRNA of an internal control gene; and
 - f) determining a chemotherapeutic regimen for treating the metastatic tumor in the individual based on the amount of ~~tumor gene determinant~~ EGFR mRNA in the amplified sample and a predetermined threshold level for ~~the tumor gene determinant~~ EGFR.
25. (currently amended): A method for determining a chemotherapeutic regimen for treating a metastatic tumor in an individual having a primary and metastatic tumor, comprising
- a) obtaining a primary tumor specimen;
 - b) fixing and paraffin embedding (FPE) the primary tumor specimen;
 - c) deparaffinizing the tumor specimen to obtain a deparaffinized sample;
 - d) determining gene expression levels for EGFR in the primary tumor specimen, comprising
 - 1) isolating mRNA from the deparaffinized sample, wherein said sample is heated to a temperature in the range of about 50°C to about 100°C; and
 - 2) determining the amount of EGFR mRNA by amplifying the mRNA using an oligonucleotide primer pair, the pair comprising a primer having SEQ ID: 1, or an oligonucleotide primer at least 80% identical therewith and hybridizes to a complement of SEQ ID NO: 1 under stringent conditions; wherein said isolated and purified oligonucleotide is capable of amplifying a portion of the 5' untranslated region and Exon 1 of EGFR mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 2, and a primer having

- SEQ ID: 2 or an oligonucleotide primer at least 80% identical therewith and hybridizes to a complement of SEQ ID NO: 2 under stringent conditions; and wherein said ~~isolated and purified~~ oligonucleotide primer is capable of amplifying a portion of the 5' untranslated region and Exon 1 of EGFR mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 1; ~~and~~
- e) comparing the amount of EGFR mRNA from step d) to an amount of mRNA of an internal control gene; and
- f) determining a chemotherapeutic regimen for treating the metastatic tumor in the individual based on the amount of EGFR mRNA in the amplified sample and a predetermined threshold level for the EGFR.
26. (previously presented): The method of claims 20 or 25, wherein the mRNA is isolated in the presence of an effective amount of chaotropic agent.
27. (currently amended): The method of claim 20, ~~23~~, 24, or 25 wherein the heating occurs at a temperature from about 75°C to about 100°C for a period of about 5 to about 120 minutes.